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Ultrastructure of the Human Dermal Microcirculation. III. The Vessels in the Mid- and Lower Dermis and Subcutaneous Fat

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This paper describes the ultrastructure of the microcirculatory vessels in the mid- and lower dermis and subcutaneous fat. Reconstruction of vessel walls, tracing out the courses of individual vessels, and survey examination of vessels were carried out by various combinations of routine and serial, ultrathin, and 1-μm sections. Intracellular myofilamentous bundles associated with extracellular filaments were a characteristic feature of the endothelial cells in arterioles possessing an internal elastic lamina, but were only rarely seen in endothelial cells of venules. The ultrastructural features of these bundles and filaments suggested both contractile and anchoring functions. The elastic lamina of the arterioles was shown to be composed of individual elastic fibers oriented in the long axis of the blood vessel under the endothelium, rather than being a continuous sheet as in larger arteries and arterioles. The capillaries in the fat had walls of normal thickness (0.1–0.3 μm) similar to capillaries in other organs, in contrast to the dermal capillaries whose walls are 2–3 μm thick. The arterioles, capillaries, and venules in the fat were frequently devoid of veil cells in contrast to those in the dermis. Venous capillaries with bridged fenestrations were found in close proximity to eccrine sweat glands and hair bulbs in the forearm, trunk, and buttock skin.

Our 2 previous reports on the ultrastructure of the cutaneous microcirculation dealt with the capillary loops in the dermal papillae, and the arterioles, capillaries and postcapillary venules in the superficial horizontal plexus [1,2]. We showed that there was ultrastructural differences between the arterial and venous sides of the microcirculation. In the arterial segments, the basement membrane material in the vascular wall had a homogeneous appearance, but in the venous portions of mural basement membrane material was laminated. In addition the endothelial cells of the arterial vessels had a more electron dense cytoplasm and more caveolae than venous endothelial cells. The capillary loops in the dermal papillae had arterial features without bridged fenestrations. The descending limb of the loop was wider than the ascending one.

In this paper we report our analysis of the vessels present in the mid- and lower third of the dermis, in the lower horizontal plexus situated at the junction of the fat and dermis, and within the fat lobules.

MATERIALS AND METHODS

Three-millimeter discs of normal appearing flexor forearm, thigh, lower abdomen, and buttock skin were obtained from 4 individuals with a skin trephine. An intradermal ring of anesthesia was produced with 1% lidocaine without epinephrine. The sample of skin was removed from the center of the ring and processed for light and electron microscopy by techniques previously described [1]. The ages and diagnoses of the 4 volunteers supplying specimens of their normal appearing skin were as follows: a 33-yr-old man with psoriasis—buttock skin; a 53-yr-old man with lichen simplex chronicus—flexor forearm skin; a 57-yr-old man with psoriasis—thigh skin; and a 75-yr-old woman with telangiectasia of unknown etiology on the chest and face—lower abdominal skin. The vessels in the mid- and lower third of the dermis, in the lower horizontal plexus and in the fat were examined in 2 ways. Many sections were taken at different levels in the blocks and examined in survey fashion by transmission electron microscopy. Serial 1-μm sections combined with ultra thin sections of the plastic embedded material were made in order to reconstruct the paths of individual vessels and simultaneously study the structural arrangements of the vessels along their longitudinal courses and at their branching points. The electron microscope was calibrated with a cross grating replica (Polysciences, cat. no. 7321) each time specimens were photographed. The replica was photographed at each of the magnifications used and the film was processed along with the film used to photograph the tissues. The exact enlargement of each print was calculated from a photograph of the replica printed at the same magnification on the photographic enlarger. The scale of measurement was calculated on the basis of the number of lines per mm on the replica as stated by the manufacturer. Measurements were made on prints taken in a through focus series.

These studies were approved by the Human Investigations Committee at Yale.

RESULTS

General findings

The biopsies from all 4 cutaneous sites showed identical findings. The ultrastructure of the arterioles and venules in the mid- and lower dermis and in the lower horizontal plexus differed from the ultrastructure of comparable vessels in the superficial horizontal plexus in several ways. Most of the arterioles and venules in the lower plexus were 50 μm in diameter with walls 10 to 16 μm thick (Fig 1 and 2). There were 4 or 5 layers of smooth muscle cells or pericytes respectively in their walls in contrast to 1 or 2 layers in similar vessels of the superficial plexus. In the deep dermal arterioles (>20 μm), collagen fibrils 0.05–0.1 μm in diameter and grouped in bundles of 50–100 fibrils, were oriented parallel to the long axis of the vessel in the subendothelial layer and in between the smooth muscle cells. They were embedded in the homogeneous appearing basement membrane material of the vascular wall. This pattern is different from that seen in the small arterioles (<20 μm) of the superficial plexus in which the bulk of the collagen fibrils were distributed as individual units diffusely within the peripheral zone of the vascular wall. The rest of the vascular wall contained only an occasional collagen fibril.

In the deep dermal venules, the collagen fibrils were distributed individually throughout the vascular wall parallel to the long axis of the vessel. A few collagen fibrils were present in the subendothelial layer and in between the first 2 layers of pericytes, but most of the observed fibrils were concentrated in the outer half of the vascular wall (Fig 2). Rarely, a small elastic fiber was present in the subendothelial layer. In the corresponding venules (<20 μm) of the superficial horizontal plexus, individual collagen fibrils were similarly distributed. The basement membrane material in the walls of the venules of the mid- and lower dermis and fat, had the same laminated appearance as in venules of the upper dermis. The laminated basement membrane material and the generalized distribution of collagen fibrils made the cellular and noncellular elements in the walls of the venules appear to be less compactly arranged than those

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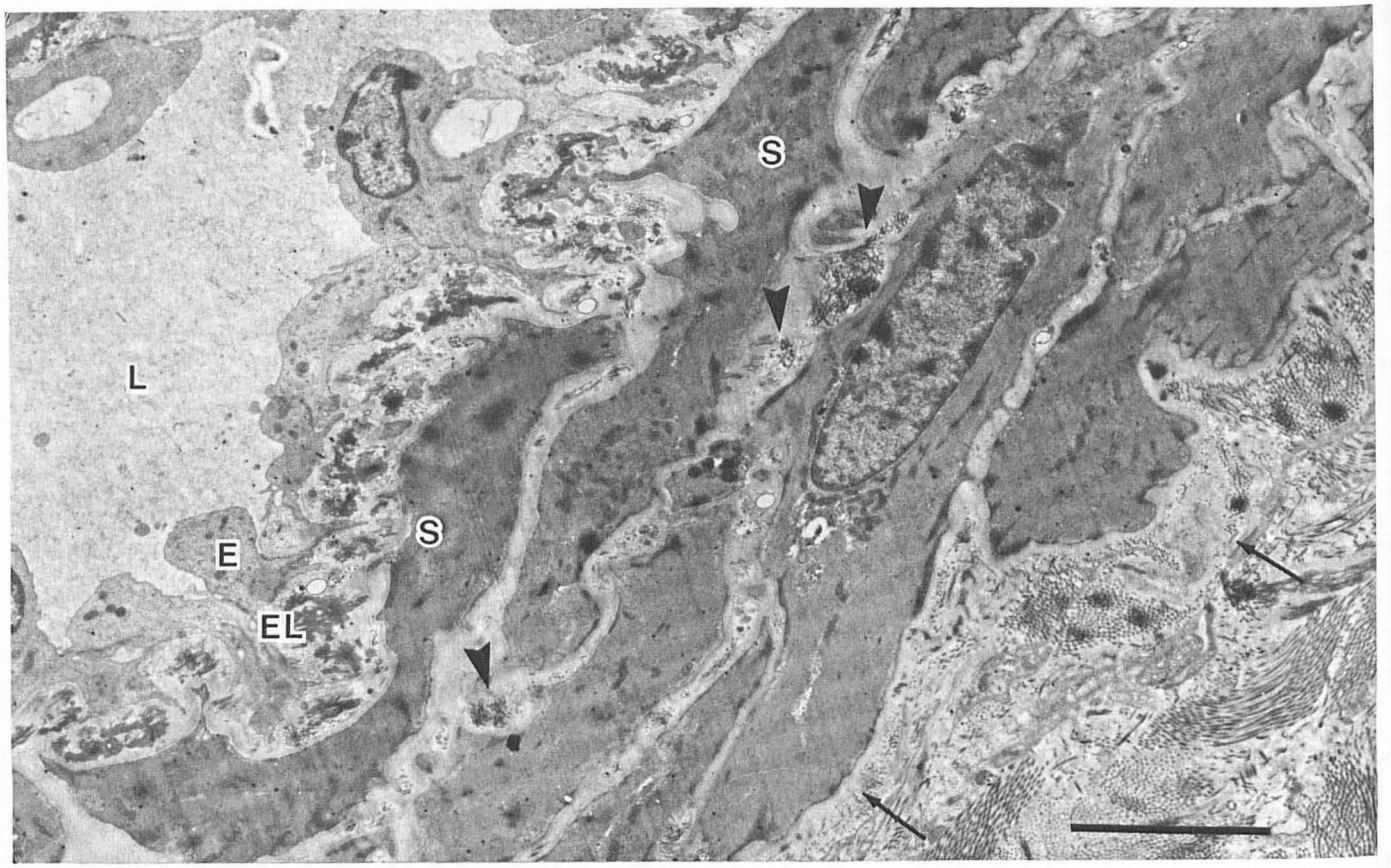

FIG 1. Arteriole in deep dermis. Portion of wall seen in cross section. *L* = lumen. *E* = endothelial cell. *EL* = elastic tissue. *S* = smooth muscle cell. *Arrowheads* indicate bundles of collagen fibers. *Arrows* indicate boundary between vascular wall and dermal collagen. *Bar* = 5 μm.

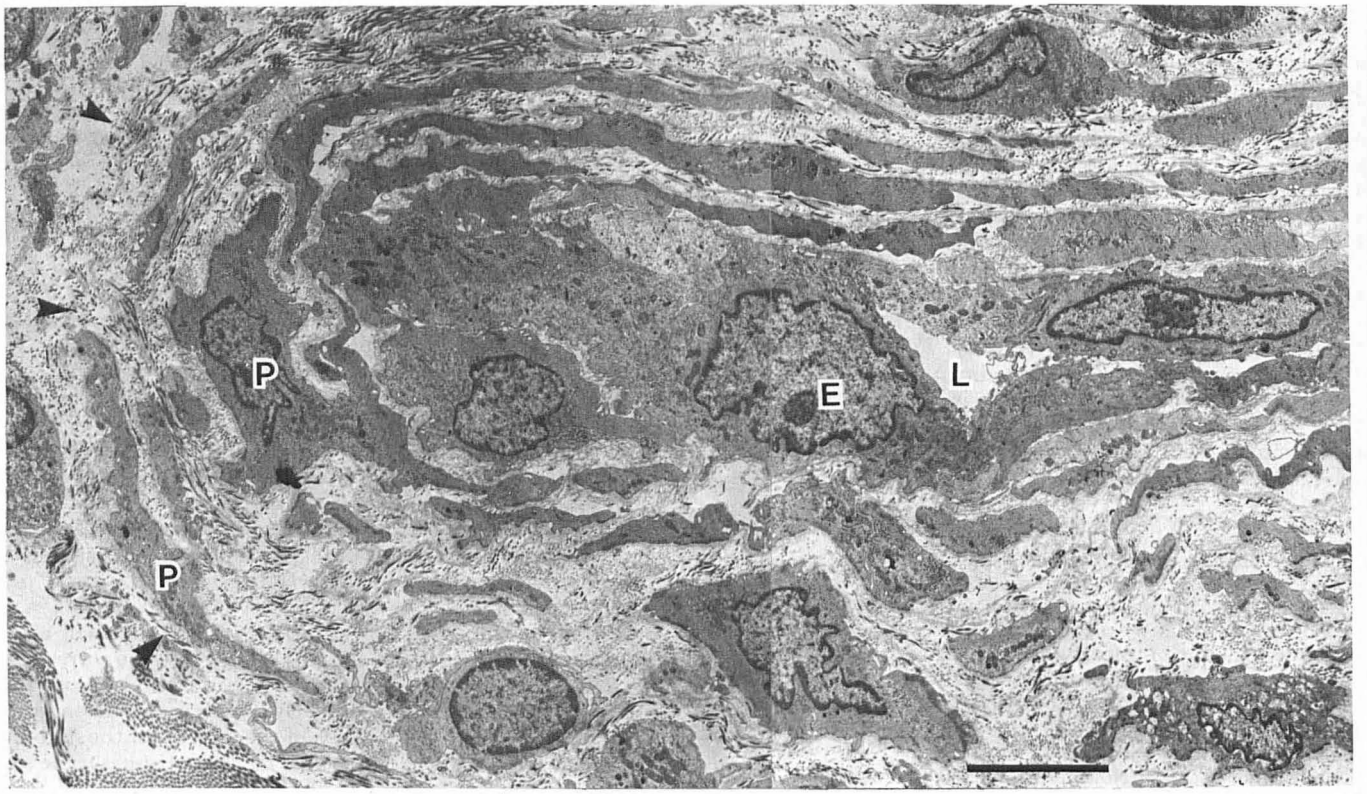

FIG 2. Venule in deep dermis. Cross section. *L* = lumen. *E* = endothelial cell. *P* = pericyte. Majority of collagen fibrils are in outer half of wall. *Arrowheads* indicate boundary between wall and dermis. *Bar* = 5 μm.

in the arterioles. The arterioles and venules in the fat lobules were identical in structure and size to those of the lower horizontal plexus. Rarely, an arteriole 100 μm in diameter was seen. The walls of the capillaries in the fat were only 0.1–0.3 μm thick in contrast to upper dermal capillaries in which the walls are characteristically 2–3 μm thick. In addition, veil cells which are flat, fibroblastlike cells that encircle all arterioles, capillaries, and venules in the dermis were not always present around the corresponding vessels in the subcutaneous layer. Venous capillaries with bridged fenestrations were found in close proximity to eccrine sweat glands and hair bulbs (Fig 3).

The arterioles with an internal elastic layer exhibited 2 unusual features:

1. The elastic fibers did not exist as a continuous band beneath the endothelium, but rather were present in discrete bundles (Fig 1) separated from one another by the cytoplasmic processes of smooth muscle cells. Frequently these processes made contact with the endothelium—an observation we had noted and illustrated in a previous report [1]. When studied in reconstructed longitudinal serial sections, the elastic layer was found to be composed of individual fibers of different lengths present at all levels between the endothelial cell and the first layer of smooth muscle cells (Fig 4). The elastic fibers were embedded in the basement membrane material of the wall. The individual elastic fibers branched and joined one another at various points to produce an irregular network (Fig 5) that was integrated into a circumferential layer. As the course of individual arterioles was followed in serial sections there was no change in the organization of the elastic layer. At points where either a smaller arteriole or an arterial capillary arose as a branch from the main trunk, the elastic tissue did not continue into these side channels. There was an abrupt disappearance of elastic fibers at the branching point; never a gradual disappearance as is characteristic of the superficial dermal elastic arterioles that give rise to the smaller nonelastic arterioles and capillaries [1]. In 2 instances, where the courses of the side branches were followed, an arteriole was observed to suddenly develop the ultrastructural features of a postcapillary venule without passing through the features of an arterial or venous capillary.

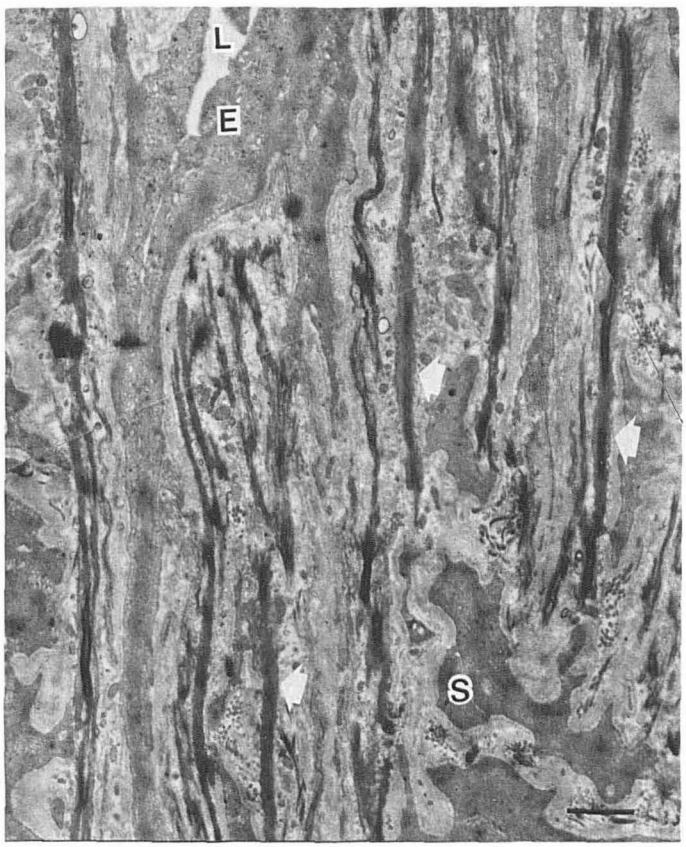

FIG 4. Arteriole in deep dermis. Longitudinal section through wall showing individual elastic fibers which are present between endothelial cells and first layer of smooth muscle. *L* = lumen. *E* = endothelial cell. *S* = smooth muscle cell. *Arrowheads* indicate elastic fibers. *Bar* = 1 μm.

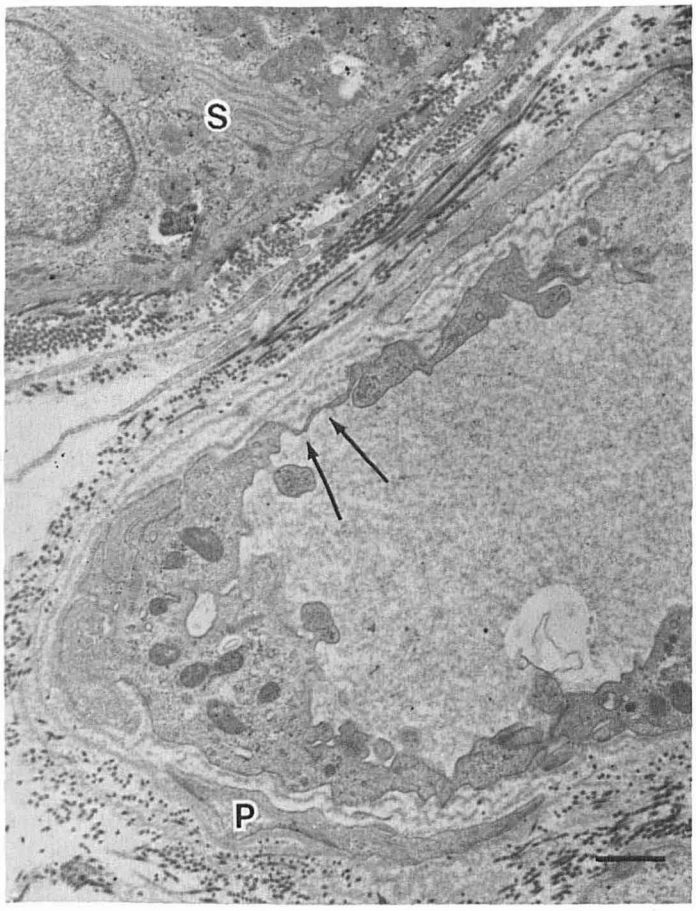

FIG 3. Venous capillary in deep dermis adjacent to eccrine gland. *S* = eccrine gland. *Arrows* indicate area of bridged fenestration in endothelial cell. *P* = pericyte. Basement membrane material in vascular wall is laminated. *Bar* = 1 μm.

2. The endothelial cells of the arterioles in the mid- and lower dermis and fat contained bundles of 4–7 nm (mean 5.2 nm) filaments that tended to course along the abluminal border of the cells. These bundles exhibited transverse linear wavy bands of increased density resembling the Z bands of skeletal muscle (Fig 6 and 7). The bands occurred at intervals varying from 0.3 to 0.8 μm. The bundles themselves varied in width from 0.5 to 1 μm. The bands of increased density represented areas where the filaments were more closely aggregated than in the rest of the bundle. In longitudinal sections, these intracellular filaments appeared to be associated with extracellular bundles composed of filaments measuring 10–20 nm in diameter (Fig 6–9). In some sections there was a dense linear zone in the region of the cell membrane where the intracellular filaments and extracellular filaments met (Fig 6–8); however we were not able to resolve the internal structure of this dense area. The extracellular filaments were observed to form 4 kinds of linkages. They formed a connection between the intracellular bundles of 2 adjacent endothelial cells (Fig 6); in endothelial cells with an irregular abluminal border, they linked one intracellular bundle with another in the same cell after coursing through the underlying basement membrane material (Fig 8); and they also appeared to run from the intracellular bundle to the underlying

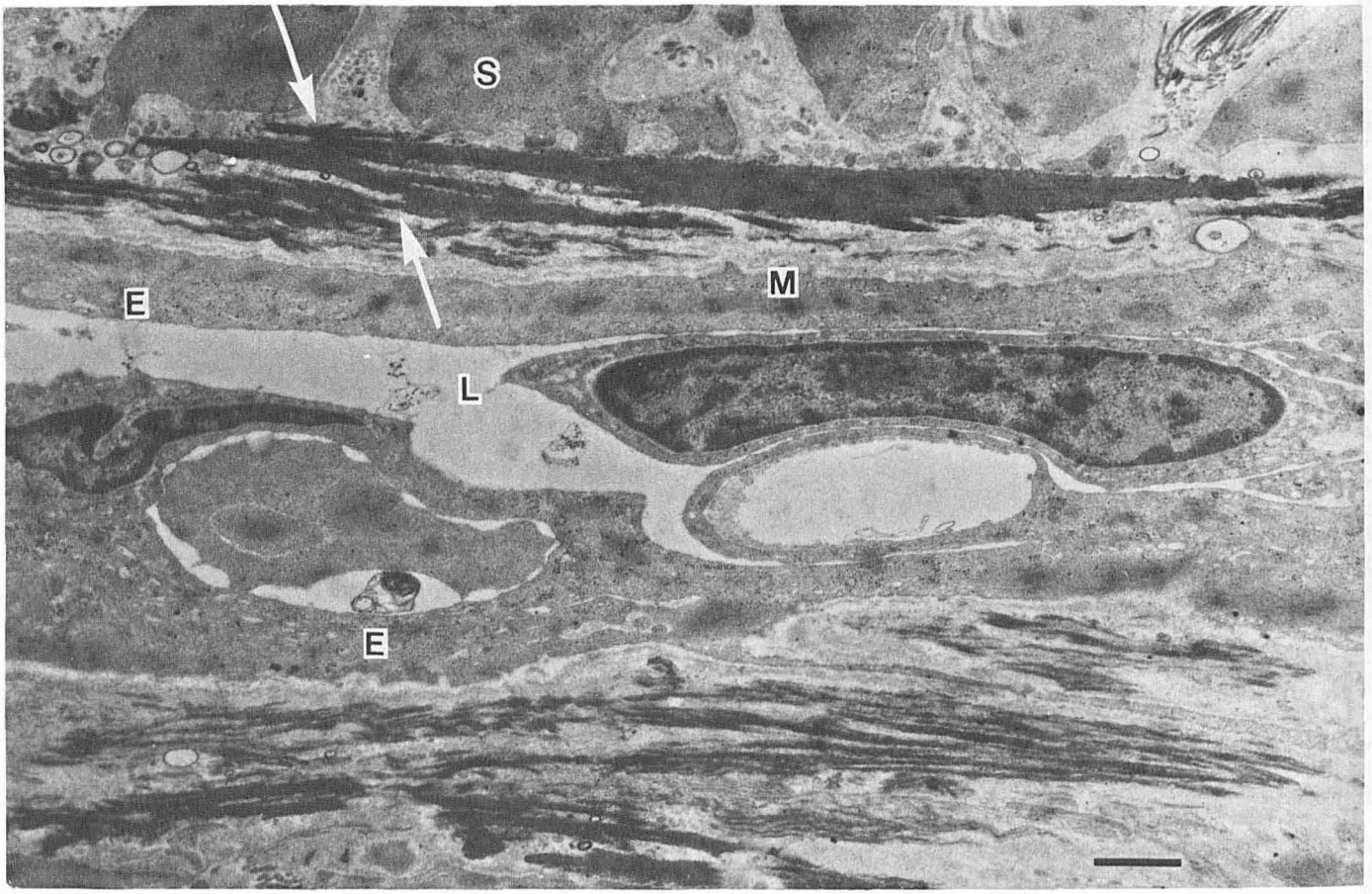

FIG 5. Arteriole in deep dermis. Longitudinal section. Individual elastic fibers branch and join one another to form an irregular network (*arrows*). *L* = lumen. *E* = endothelial cell. *S* = smooth muscle cell. *M* = intracellular banded bundle. *Bar* = 1 μm.

mural basement membrane material (Fig 8) or to an adjacent elastic fiber (Fig 9). The extracellular filaments appeared to connect with an adjacent elastic fiber through the latter's microfibrillar component (Fig 9). In cross sections of endothelial cells, these intracellular bundles appeared as oval to linear densities along the abluminal border. The extracellular filaments were seen below them, both in cross section and in tangential profiles (Fig 10). We did not see the complex of intracellular and extracellular filaments in elastin-free arterioles or in postcapillary venules of the superficial plexus. The complex was only rarely seen in the large venules (50 μm) of the deep dermis and subcutaneous layer.

DISCUSSION

The arterial and venous capillaries in the fat differ from those in the upper and lower dermis by having walls only 0.1–0.3 μm thick, thus resembling capillaries in other organs. (The capillaries in the dermis have walls 2–3 μm thick). The veil cells which are so prominent around all the microcirculatory vessels in the dermis are not found as often around these same vessels in the subcutaneous layer.

The internal elastic lamina in arteries 350 μm and larger is a continuous sheet of elastic tissue present beneath the endothelial layer [3]. The lamina is perforated so that the underlying smooth muscle cells can insinuate a portion of their cytoplasm to make junctional contact with the endothelial cells. However, in the smallest to the largest dermal and subcutaneous arterioles observed (50–100 μm), the internal elastic layer was not a continuous sheet but was composed of individual elastic fibers, frequently cross linked to their neighbors to produce a net-like sheath. The network of elastic fibes which was oriented parallel to the long axis of the vessel was embedded in the basement membrane material of the wall, analogous to reinforcing steel rods in concrete. In our earlier studies in which we traced the course of arterioles through the capillary bed to their venous connections, we had observed that the elastic fibers which were initially located beneath the endothelium became more peripherally situated in the wall as the individual arterial vessel became smaller [1]. The elastic tissue eventually disappeared and formed a discontinuous ring outside the wall, disappearing just before the vessel developed the features of a capillary. The external ring of elastic fibers visualized by electron microscopy represents part of the network of elastic fibers that can be demonstrated around the upper dermal arterioles in paraffin embedded sections stained for elastin. Based upon our current findings, this portion of the external network appears to emerge from the vessel as a continuation of the subendothelial and peripherally placed elastic network found in arterioles up to 20 μm in diameter. The deep dermal arterioles did not show this pattern of elastic fiber deployment either along their course or at their branching points. The elastic fibers remained in a subendothelial position throughout the examined length of the 50–100 μm arterioles.

The major difference between the deep arterioles in the fat and lower dermis and the arterioles of the papillary dermis is the presence of a complex of intracellular and extracellular filaments. The intracellular component of 5.2 nm filaments which form an irregularly banded bundle resembling skeletal muscle has been described previously in endothelial cells from the femoral artery of the rat [3], splenic artery and sinusoids of man and rat respectively [4,5], human umbilical and endometrial arteries [6,7], and the myometrial artery of the rat [8]. However in none of these studies were extracellular filaments described. Yohro and Burnstock also described endothelial intracellular bundles in the coronary arteries of several different animals, including man [9]. They found short extracellular fibrils that passed from the underlying continuous elastic lamina to the endothelial cell membrane where the intracellular

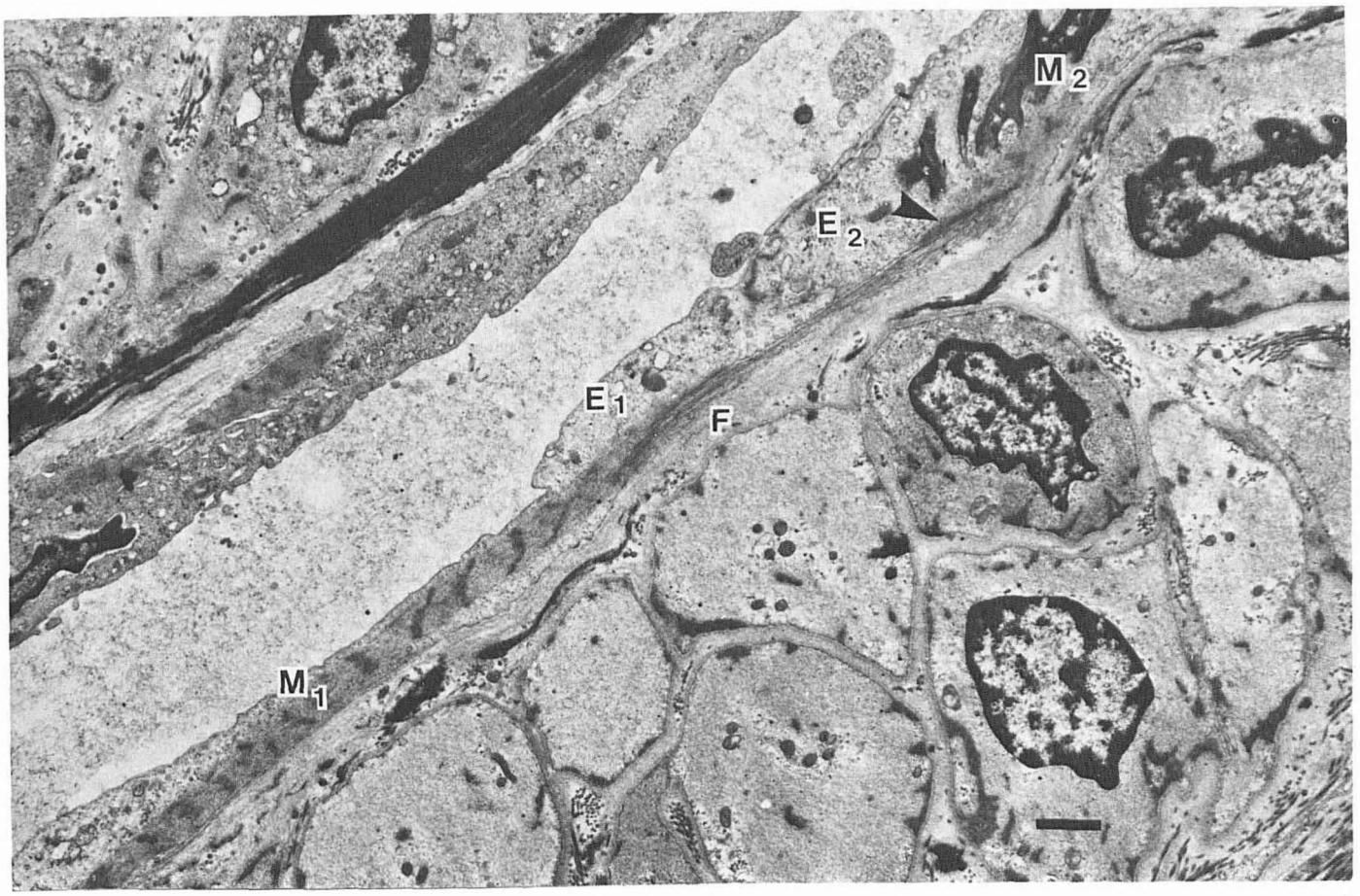

FIG 6. Arteriole with banded intracellular bundles. Longitudinal section. Intracellular bundle (M_1) connected to bundle (M_2) in adjacent endothelial cells (E_1 *and* E_2) by extracellular filaments (F). *Arrowhead* indicates dense linear zone in region of cell membrane where intercellular and extracellular filaments meet. *Bar* = 1 μm.

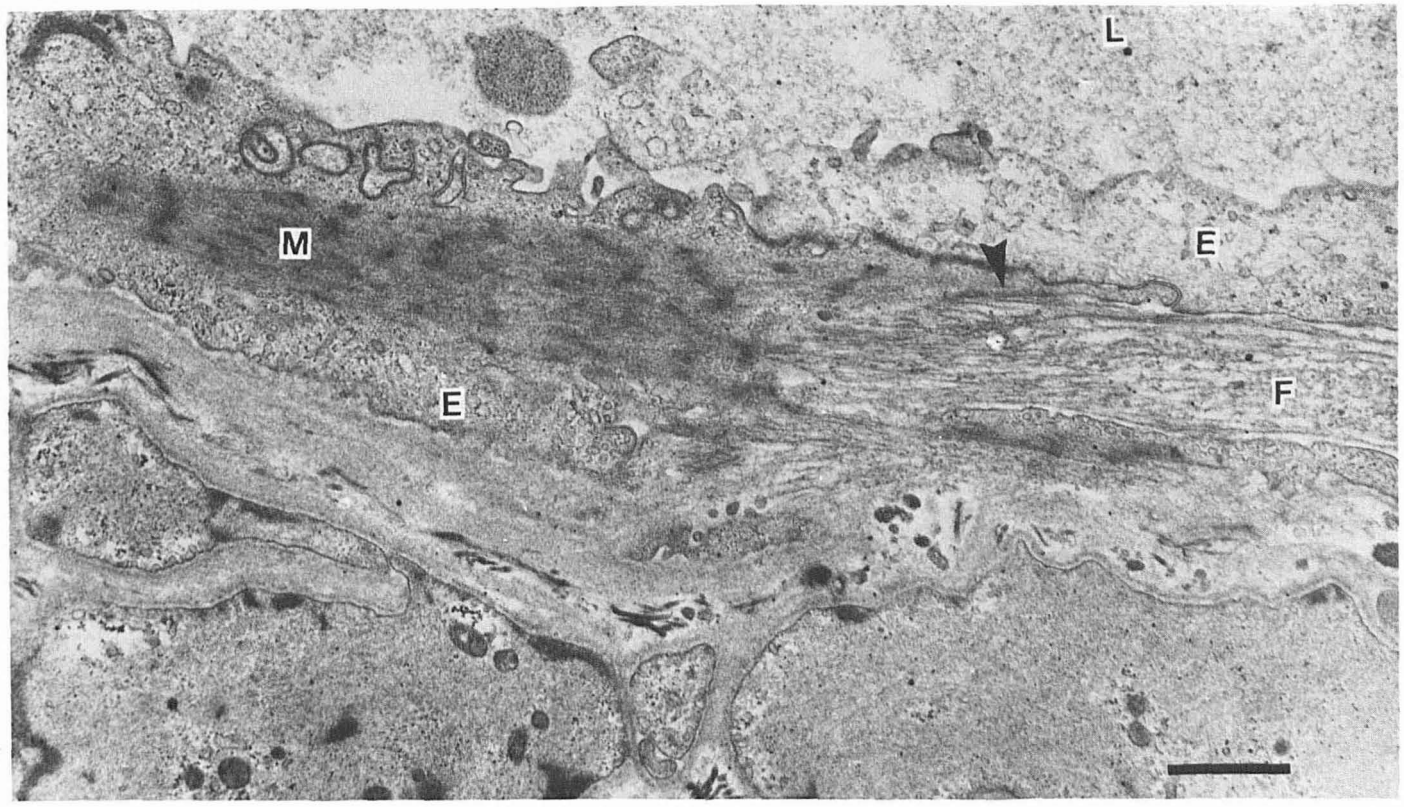

FIG 7. Arteriole in deep dermis. Longitudinal section. M = intracellular bundle with irregular banding. F = extracellular filaments. E = endothelial cell. L = lumen. *Arrowhead* indicates dense linear zone where intra- and extracellular filaments meet. *Bar* = 1 μm.

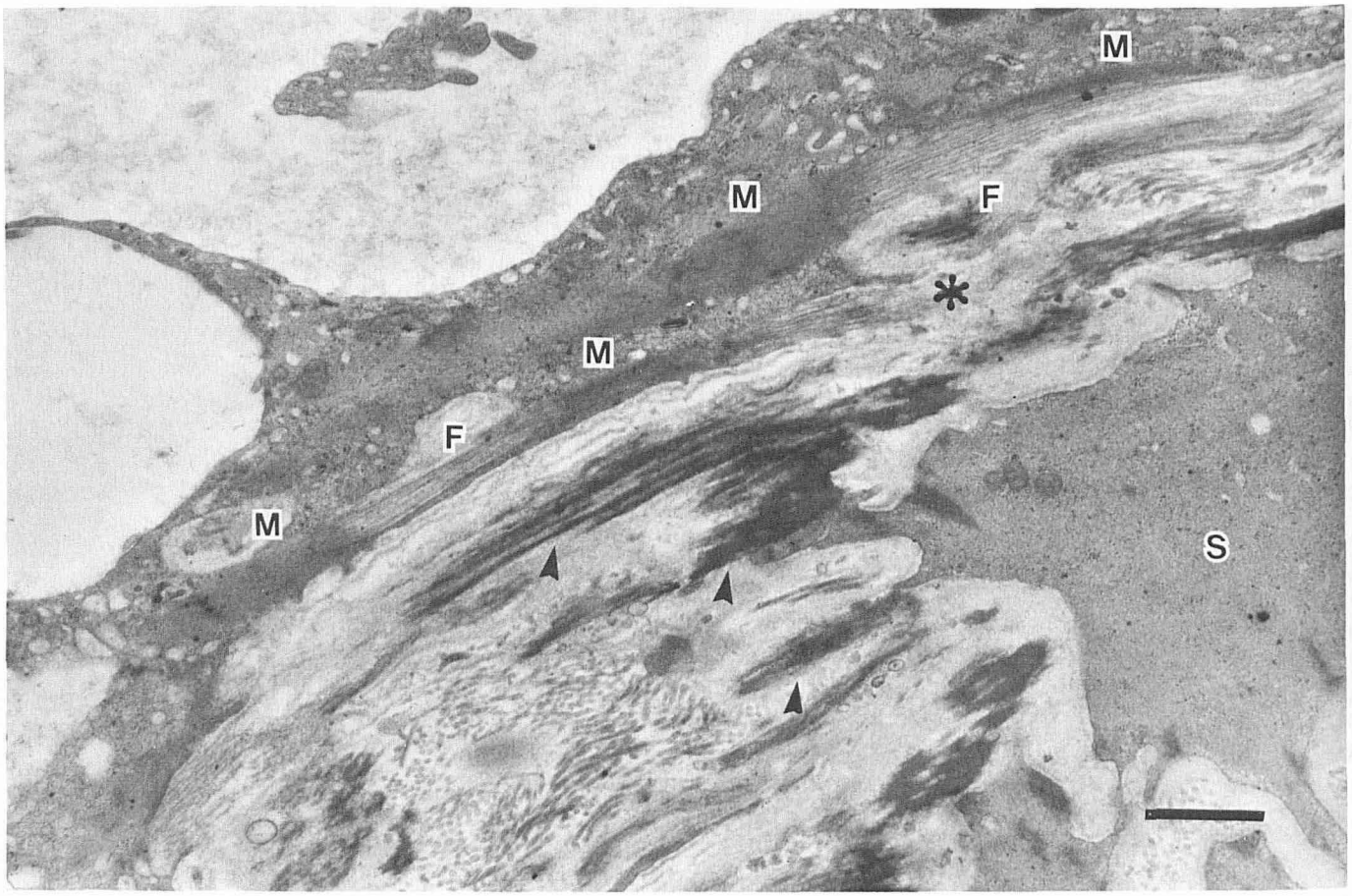

FIG 8. Arteriole in deep dermis. Longitudinal and slightly tangential section. *M* = intracellular bundle. *F* = extracellular filaments connecting intracellular bundles in same endothelial cell (*E*). *S* = smooth muscle cell. *Arrowheads* indicate elastic fibers. *Asterisk* indicates connection of extracellular filaments with basement membrane material. *Bar* = 1 μm.

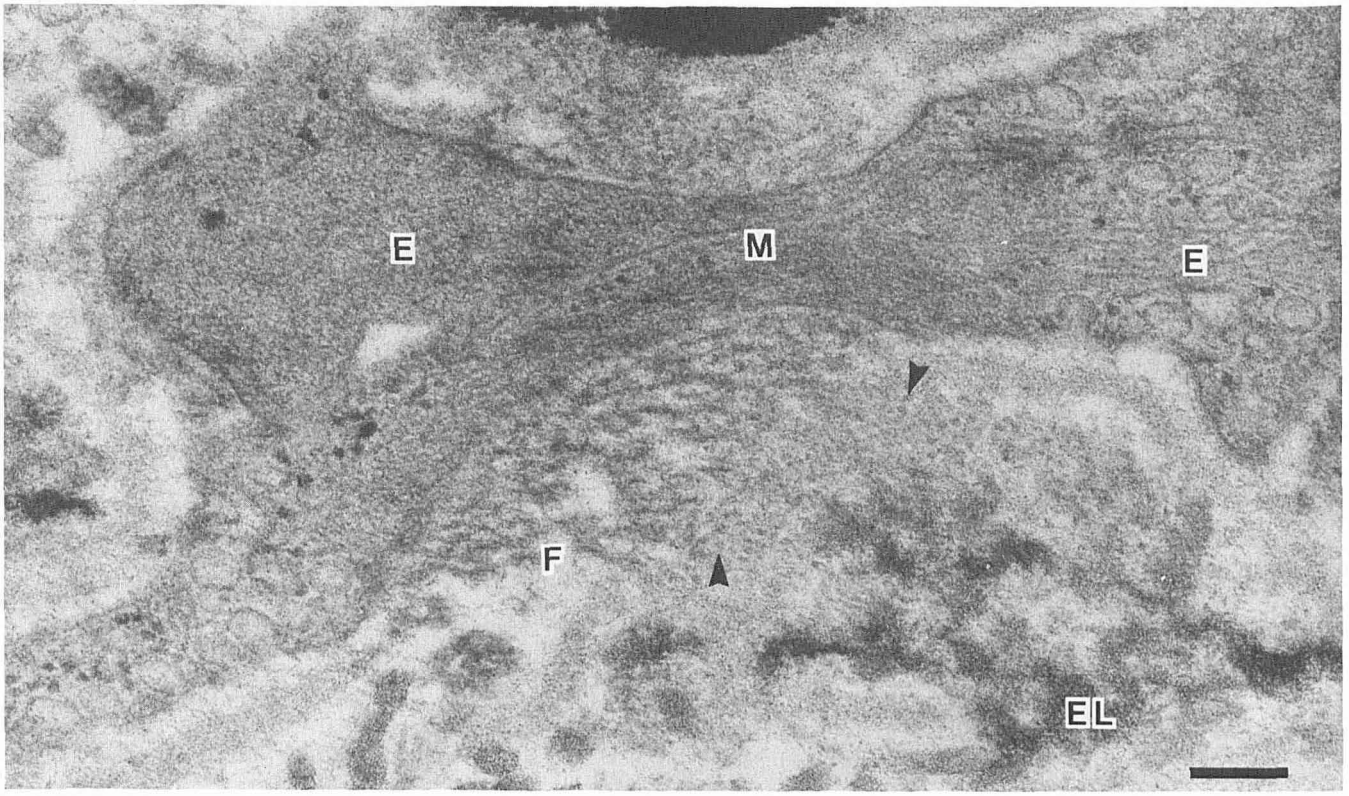

FIG 9. Arteriole in deep dermis. Cross section. External filaments (*F*) are terminating in microfibrillar component (*arrowheads*) of elastic fiber (*EL*). In this section, the intracellular bundle appears as a slightly increased linear density without banding. *E* = endothelial cell. *Bar* −0.2 μm.

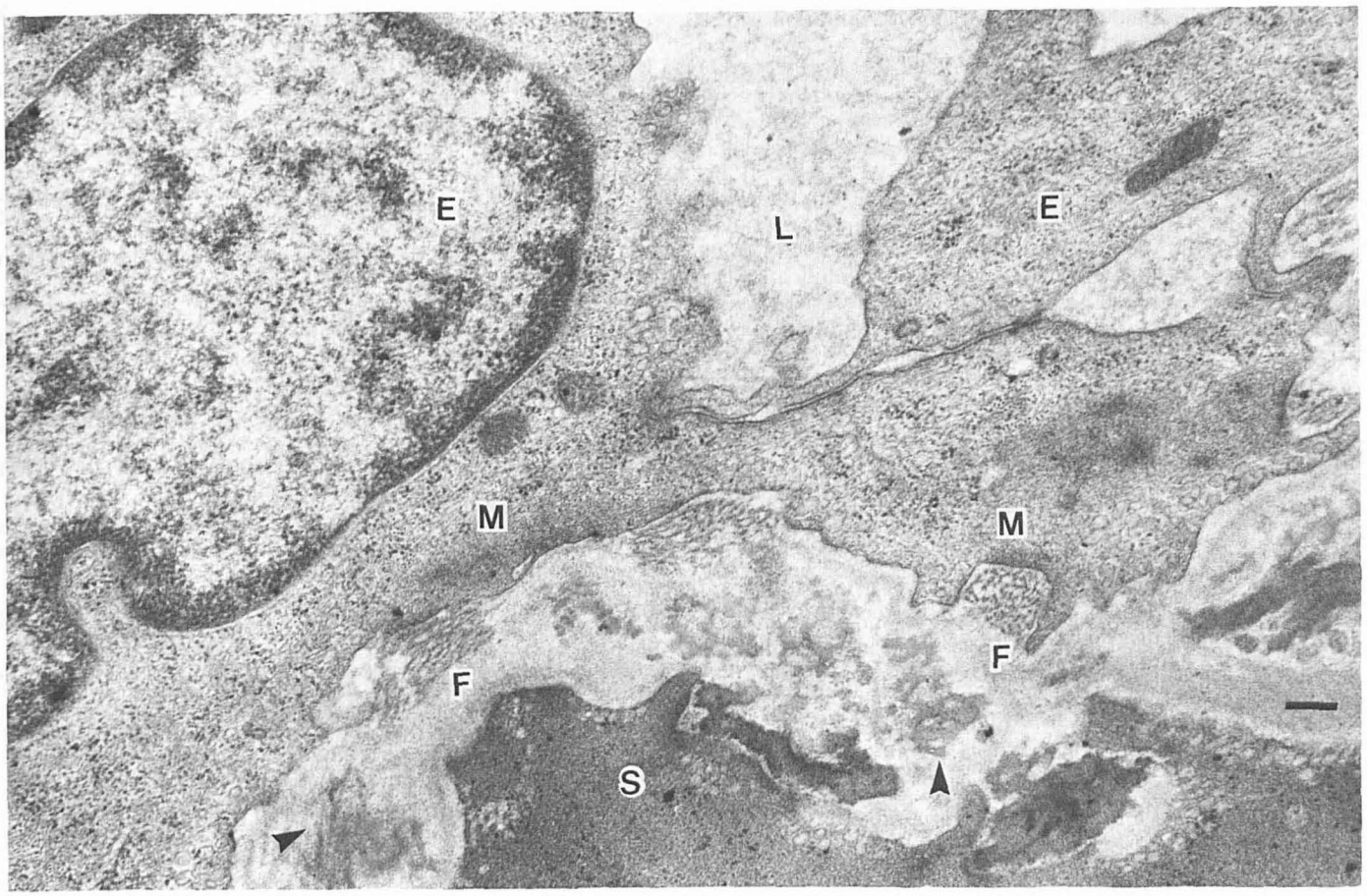

FIG 10. Arteriole in deep dermis. Cross section. Intracellular bundles (*M*) appear as linear densities along abluminal border of endothelial cell (*E*). Extracellular filaments (*F*) are present in cross sectional and tangential profiles. *S* = smooth muscle cell. *L* = lumen. *Arrowheads* indicate elastic fibers. *Bar* = 0.2 μm.

bundles appeared to insert. Yohro and Burnstock believed the connecting fibrils were elastic microfibrils.

The filament complex we describe is different from that reported by Yohro and Burnstock. The external component is more prominent, perhaps because the subendothelial zone of dermal arterioles is not a narrow band completely filled with a continuous elastic sheet, but instead is a broad belt composed of basement membrane material in which are embedded elastic fibers and small bundles of collagen fibrils. The external filaments are longer and larger (10–20 nm) than those reported by Yohro and Burnstock and they form a variety of linkages. In some, the free ends of the extracellular filaments were observed to insert into the basement membrane material of the vascular wall or into elastic microfibrils present on the margins of elastic fibers. The external filaments also made connections between portions of the same cell or with adjacent cells after traversing the basement membrane material of the vascular wall.

The 5 nm filaments found in endothelial cells of blood vessels and lymphatics are thought to be actin. Actin has been identified in such filaments by decoration with heavy meromyosin [10–12]. Actomyosin and myosin have also been identified in endothelial cells by immunologic techniques [13,14]. Based upon these findings, it seems likely that the endothelial 5.2 nm filaments in dermal arterioles, which are organized into bundles resembling myofilaments, do have a contractile function. The 5.2 nm filaments are to be distinguished from the 10 nm filaments which are also present in the cytoplasm of endothelial cells and which are believed to function as a cytoskeleton [1,11].

The nature of the extracellular filaments is not known. However the available experimental data suggest the following possibilities. The endothelial cells of lymphatic capillaries have anchoring filaments which are morphologically similar to elastic microfibrils [15]. Jaffee et al demonstrated by electron microscopy that endothelial cells from blood vessels in culture appeared to synthesize material closely resembling elastic microfibrils and elastin [16]. Singer studied a complex of intracellular and extracellular filaments found in fibroblasts in culture which resembles that seen in the dermal arterioles described in this report [17]. The extracellular fibers which measured 5–20 nm, contained fibronectin and were colinear with intracellular 5 nm filaments. However, the intracellular filaments did not form cross banded bundles. The 5 nm intracellular filaments were believed to be actin because of their size and continuity with the stress fibers of fibroblasts which are actin rich. The external and internal filaments joined together in a submembranous plaque that stained densely with tannic acid. Singer called this complex a fibronexus. Since endothelial cells have been shown to synthesize fibronectin [18] and blood vessels contain fibronectin in the basement membrane of the endothelium [19], it is possible that the filament complex in cutaneous arterioles represents an actin—fibronectin enriched complex (fibronexus). The extracellular filaments may be fibronectin coated elastic microfibrils.

The combination of the external and internal filaments in dermal arterial endothelial cells may serve 2 purposes: a contractile function because the 2 sets of filaments have a morphologic relationship similar to a muscle and its tendon; and an anchoring function based on the manner in which the external filaments traverse the basement membrane material of the vascular wall as they pass from one cell to another, or back to the same cell. The termination of external filaments in the mural basement membrane material and on adjacent elastic fibers also support an anchoring function. Occasionally, we observed 10–20 nm external filaments in the absence of intracytoplasmic bundles passing from the endothelial cells of deep

dermal and subcutaneous venules to their termination in the basement membrane of the vascular wall.

Although the external and internal filaments in the dermal arterioles appeared to be colinear, we did not conduct precise measurements to establish this point, nor were we able to determine the exact site and manner by which the external and internal filaments seemed to be associated.

The intracellular myofilamentous bundles of 5 nm filaments have been found almost exclusively in arteries and arterioles. Majno et al found only 2 examples of these bundles in 1,000 pictures of venules [20]. In a study of human umbilical vessel endothelium, Parry and Abramovich found these bundles in both umbilical veins and arteries during the first 10 weeks of gestation, but only in the arteries after this time [6].

Because of the longitudinal orientation of the intracellular bundles, endothelial cell contraction in arterioles would produce a shortening of the cell with a bulging of the nucleus into the lumen impeding blood flow. Relaxation of the cell would increase luminal diameter and thereby increase flow. Is it possible that the smooth muscle in the arteriolar wall is a coarse regulator and the intracellular bundles a fine regulator of blood flow?

Fenestrated capillaries are found in areas where there is a need for rapid exchange of molecules between the vascular system and the tissues. They are believed to be the morphological equivalent of the large pore system originally proposed by physiologists [21]. Fenestrated capillaries are found in renal glomeruli, endocrine glands, intestinal lamina propria, the choroid plexus of the brain and the ciliary body of the eye. In the skin, we have observed them around the sweat glands, hair bulbs, and in the dermal papillae of psoriatic lesions. McLeod also found them in, and immediately around, the papillae of the hair bulbs on the scalp [22].

The cutaneous microcirculatory blood vessels are unique. They have extremely thick walls, a highly developed system of endothelial cell-related external filaments that most likely have an anchoring function, and a distinctive internal and external pattern of elastic fiber deployment. All 3 features would be protective against the constant external shearing forces to which these vessels are subjected. The differentiation between the arterial and venous sides of the microcirculation based on the appearance of the basement membrane material holds true for all vessels from the superficial dermis to the fat. This ultrastructural distinction between the arterial and venous segments of the microcirculation has been confirmed by Higgins and Eady [23]. The deep dermal arterioles and venules can also be distinguished from one another both in normal tissues as well as in pathologic states, such as in the microangiopathy of diabetes, by the distribution pattern of collagen fibrils in the vascular wall. There are now sufficient morphologic criteria available by which the vascular abnormalities in cutaneous disorders can be deciphered.

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